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A Direct Link between Sister Chromatid Cohesion and Chromosome Condensation Revealed through the Analysis of *MCD1* in S. cerevisiae

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Summary

The S. cerevisiae *MCD1* (mitotic chromosome determinant) gene was identified in genetic screens for genes important for chromosome structure. *MCD1* is essential for viability and homologs are found from yeast to humans. Analysis of the *mcd1* mutant and cell cycle–dependent expression pattern of Mcd1p suggest that this protein functions in chromosome morphogenesis from S phase through mitosis. The *mcd1* mutant is defective in sister chromatid cohesion and chromosome condensation. The physical association between Mcd1p and Smc1p, one of the SMC family of chromosomal proteins, further suggests that Mcd1p functions directly on chromosomes. These data implicate Mcd1p as a nexus between cohesion and condensation. We present a model for mitotic chromosome structure that incorporates this previously unsuspected link.

Introduction

During mitosis, sister chromatids are paired and condensed. They form bipolar attachments to spindle microtubules emanating from opposite poles. At the onset of anaphase, commonly termed the metaphase-to-anaphase transition, a series of closely timed events ensue. The cohesion between sister chromatids is synchronously dissolved on all chromosomes. Sister chromatids move away from each other by microtubule-dependent movement toward the poles. At telophase, the segregated chromosomes decondense. Both sister chromatid cohesion and chromosome condensation are essential for proper chromosome segregation.

Insights into the timing, distribution, and mechanism of sister chromatid cohesion have been provided through studies of mammalian and yeast cells. Classical cytological analyses and fluorescent in situ hybridization (FISH) revealed that in yeast and mammals sister chromatids are associated along their lengths from the time of replication until anaphase (Wilson, 1925; Selig et al., 1992; Guacci et al., 1994). In many eukaryotes, the most persistent cohesion occurs at heterochromatin associated with large blocks of repetitive DNA (Lica et al., 1986; Cooke et al., 1987; Sumner, 1991). In budding yeast, chromatid cohesion occurs despite the absence of repetitive DNA. Yet, recent studies suggest that budding yeast has heterochromatin (reviewed in Grunstein, 1997). Therefore, regions of heterochromatin, and not simply the presence of repetitive DNA, may be important sites for cohesion. Two general mechanisms for cohesion have been proposed: a persistent catenation of sister DNA molecules or a protein-mediated scaffold (Murray and Szostak, 1985; Cooke et al., 1987; Rattner et al., 1988). In budding yeast, sister chromatids of minichromosomes remain associated in the absence of catenation, supporting the idea that proteins mediate sister chromatid cohesion (Guacci et al., 1994).

The proteins comprising the structural components responsible for cohesion during mitosis have not yet been identified. INCENP and CLiP proteins are intriguing candidates since they localize between paired sister chromatids prior to anaphase in mammalian cells (Cooke et al., 1987; Rattner et al., 1988). However, their importance to chromatid cohesion is not known. The Cut2 and Pds1 proteins of fission and budding yeast, respectively, have been implicated in cohesion (Cohen-Fix et al., 1996; Funabiki et al., 1996; Yamamoto et al., 1996b). However, they are not likely to be chromosomal components that play a structural role in cohesion. For example, the Cut2 protein localizes to the mitotic spindle, not to chromosomes, and Cut2 mutants do not exhibit precocious sister separation (Funabiki et al., 1996). While *pds1* mutants do precociously separate sister chromatids, additional experiments suggest that Pds1p plays a more general role in regulating the metaphase-to-anaphase transition as well as other aspects of cell cycle progression (Yamamoto et al., 1996b).

Mammalian and yeast chromosomes undergo cell cycle–dependent chromosome condensation (Wilson, 1925; Lawrence et al., 1988; Guacci et al., 1994). A breakthrough in elucidating the mechanism of condensation was the discovery of the SMC protein family (structural maintenance of chromosomes). This family is conserved from bacteria to humans with multiple members present in each eukaryote (reviewed in Koshland and Strunnikov, 1996). SMC proteins localize to chromosomes, and a subset are essential for chromosome condensation in vitro and in vivo (Chuang et al., 1994; Hirano and Mitchison, 1994; Saitoh et al., 1994; Saka et al., 1994; Strunnikov et al., 1995). Recent data from yeast, Xenopus, and mammals suggest that SMC proteins are components of higher order complexes (Castano et al. 1996; Jessberger et al., 1996; Hirano et al., 1997). The biochemical function of SMC or SMC-associated proteins has not been determined, but it is likely that SMC complexes possess multiple activities by analogy to replication complexes. Interestingly, SMC proteins may serve a more global role in DNA metabolism since they have been implicated in dosage compensation and mitotic recombination repair (Chuang et al., 1994; Jessberger et al., 1996).

The development of FISH to monitor mitotic chromosome structure and the identification of proteins such

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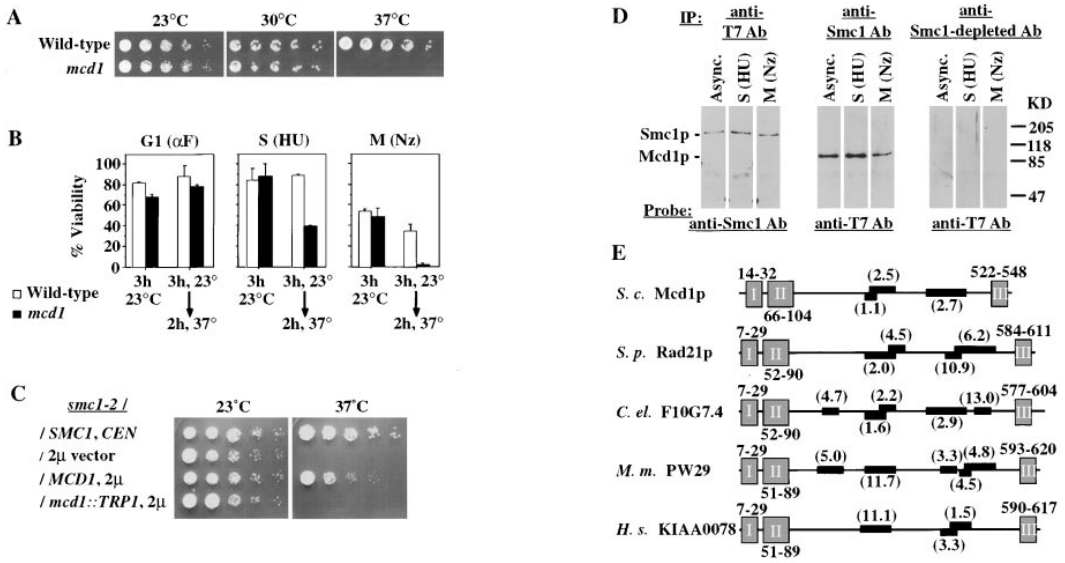

Figure 1. Characterization of the *mcd1–1* Mutant and the *MCD1* Gene

(A) Ts⁻ phenotype of *mcd1–1* cells. Wild-type (VG906–1A) and *mcd1* (VG955–7D) cells grown at 23°C in YPD liquid were plated in 10-fold serial dilutions on YPD and incubated at 23°C, 30°C, and 37°C. (B) Cell cycle–dependent lethality of *mcd1–1* cells. Strains in (A) were arrested at 23°C in either G1 (αF), S (HU), or M (Nz) phase, incubated at 37°C while arrested, then plated on YPD at 23°C to determine percent viability. Data from two independent experiments was used to generate error bars. (C) Suppression of *smc1–2* Ts⁻ phenotype by multiple copies of *MCD1*. *smc1–2* (3aAS273) cells bearing either plasmid pAS140 (*SMC1*, CEN vector), pRS426 (2μ vector), pAS271/3 (*MCD1*, 2μ vector), or pAS333 (*mcd1::TRP1*, 2μ vector) were grown at 23°C, then plated at 23°C and 37°C as described in (A). (D) Coimmunoprecipitation of Mcd1p and Smc1p. Mcd1p was overexpressed in strain BS334/pAS339, and cells either left untreated (Async), arrested in S (HU), or M (NZ) phase, then processed for immunoprecipitation. Proteins were immunoprecipitated (IP) using either anti-T7 Ab, anti-Smc1p Ab, or anti-Smc1p depleted Ab. IP proteins were Western blotted using anti-Smc1p Ab to detect Smc1p and anti-T7 Ab to detect T7 tagged Mcd1p. (E) *MCD1* is a member of a conserved gene family. Multiple sequence alignment of Mcd1p, S. pombe Rad21 protein (S.p. Rad21p) (Birkenbihl and Subramani, 1992), C. elegans ORF F10G7.4, accession U40029 (C.el. F10G7.4); mouse protein PW29 (M.m. PW29) (Yu et al., 1995), human ORF from KIAA0078 cDNA (H.s. KIAA0078) (Nomura et al., 1994) using the Pileup program of the GCG package. Boxes I, II, and III are the regions of highest shared similarity. Black bars are PESTsequences with PEST-FIND scores in parentheses.

as Pds1p and the SMC family have established budding yeast as a model system to study sister chromatid cohesion and condensation (Guacci et al., 1993, 1994; Strunnikov et al., 1995; Cohen-Fix et al., 1996; Yamamoto et al., 1996a, 1996b). The development of GFP-tagged chromosomal loci to follow cohesion has enhanced this system (Straight et al., 1996). The identification of additional components is crucial to elucidate the mechanisms of sister chromatid cohesion and condensation. Here we identify and analyze the *mcd1–1* mutant in budding yeast and show that Mcd1p is a chromosomal protein required for sister chromatid cohesion and condensation. Our analyses provide novel insights into a previously unsuspected interrelationship between cohesion and condensation.

Results

MCD1 Was Isolated by Two Screens Designed to Identify Genes Encoding Chromosomal Structural Proteins

The *mcd1* (mitotic chromosome determinant) mutant was identified in a screen to isolate mutants defective in sister chromatid cohesion (Guacci et al., 1993). To enrich for mutants defective in mitotic functions, mutants temperature-sensitive for growth (Ts⁻) were screened for enhanced inviability after arrest in M phase as compared to G1 phase. *mcd1–1* was one mutant with these phenotypes (Figures 1A and 1B). Similar mitotic lethality has been observed for cells defective in the mitotic checkpoint (Hoyt et al., 1991; Li and Murray, 1991). However, *mcd1* cells have a functional mitotic checkpoint since nocodazole-treated *mcd1* cells do not undergo new rounds of DNA replication or new bud formation (data not shown). Therefore, the mitotic lethality of *mcd1–1* cells is not due to a defect in cell cycle regulation but reflects a potential role for Mcd1p in chromosome cohesion. The *mcd1* mutant was subsequently shown to exhibit precocious dissociation of sister chromatids (see below). The *MCD1* gene was cloned by complementation of the Ts⁻ and mitotic lethal phenotypes of *mcd1* cells.

MCD1 was also identified in a screen for proteins that interact with the Smc1 protein (Smc1p), a member of the SMC family. To this end, high copy suppressors of the Ts⁻ phenotype of an *smc1* mutant (*smc1–2*) were isolated. One plasmid suppressed the Ts⁻ phenotype and associated morphological defects (Figure 1C). The suppressor gene was found to be *MCD1* (Experimental Procedures). While multiple copies of *MCD1* were required to suppress the *smc1–2* defects, they did not suppress the Ts⁻ phenotype of either an *smc1* deletion mutant or an *smc2* mutant (data not shown). These results indicate that *MCD1* suppression is specific and occurs by augmenting *smc1–2* function rather than by replacing it.

The genetic interaction between *MCD1* and *SMC1* suggested an in vivo physical interaction between the

Mcd1 protein (Mcd1p) and Smc1p. To test this possibility, a functional full-length Mcd1p tagged with the T7 epitope (Novagen) was overexpressed in an otherwise wild-type strain. Overexpression of Mcd1p recapitulates the conditions under which *MCD1* and *SMC1* genetically interact (see above). Mcd1p and Smc1p were found to coimmunoprecipitate in a chromatin-independent manner since large chromatin fragments were removed by high-speed centrifugation (Figure 1D) and coimmunoprecipitation was unaffected by DNase treatment. Polyclonal anti-Mcd1p antibodies failed to immunoprecipitate Mcd1p when expressed under its own promoter, but in preliminary experiments Mcd1p and Smc1p cofractionate through several steps of a biochemical purification (data not shown). No immunoprecipitation of Mcd1p or Smc1p was observed in strains lacking a T7 epitope tag. Taken together, these data suggest that Mcd1p and Smc1p are in a common complex.

MCD1 Is Essential and Encodes a Member of a Conserved Protein Family

MCD1 was shown to be essential by two approaches. First, a diploid strain heterozygous for a complete deletion of *MCD1* was constructed and sporulated. All tetrads contained two viable and two inviable spores. None of the viable spores contained the deleted *MCD1* gene. Second, strains were constructed in which the sole source of Mcd1p was from an *MCD1* gene under control of an inducible *GAL1* promoter. These strains were inviable in the absence of inducing agent (galactose) for *MCD1* (data not shown). Thus, loss of *MCD1* is a lethal event.

MCD1 encodes a protein with a predicted molecular mass of 63 kDa. Its sequence was compared to other proteins in the database. Mcd1p shares homology with the S. pombe Rad21 protein and with putative proteins encoded by ORFs from human, mouse, and C. elegans. These proteins are 25% identical over their entire length except for the human and mouse sequences, which are 97% identical. Three blocks of >50% similarity are shared, and their spacing and relative position is conserved (Figure 1E). The rest of the predicted protein sequence is not conserved, but in the central region there are numerous potential PEST sequences, which have been proposed to target polypeptides for rapid degradation by the 26S proteasome (Coux et al., 1996; Rechsteiner and Rogers, 1996).

Mcd1p Activity Is Essential for Proper Chromosome Segregation

The mitotic lethality of *mcd1–1* cells suggested a mitotic function for Mcd1p. To determine the role of *MCD1* in cell cycle progression, wild-type and *mcd1–1* haploid cells growing at 23°C were shifted to 37°C and cell, spindle, and DNA morphologies as well as DNA content were scored. At 23°C, *mcd1* cells were indistinguishable from wild-type cells at 23°C or 37°C (Figure 2A). However, at 37°C, the *mcd1* culture was enriched for cells with 2C DNA content (Figure 2A) and showed a 3-fold increase in the frequency of large budded cells with short or partially elongated spindles.

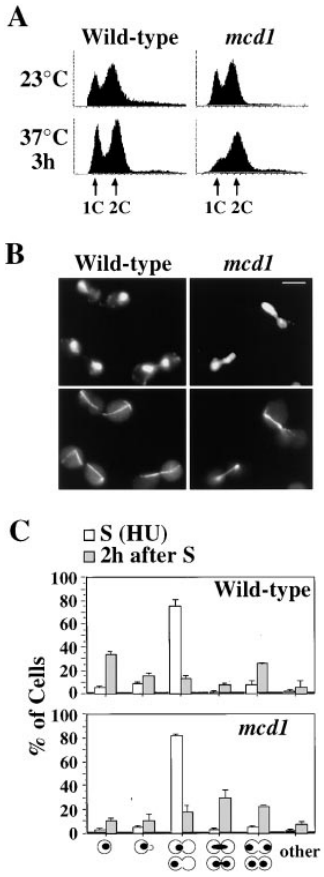

Figure 2. Cell Cycle Progression in Wild-Type and *mcd1–1* Cells

(A) DNA content of wild-type (VG906–1A) and *mcd1* (VG955–7D) cells grown asynchronously at 23°C or shifted to 37°C for 3 hr.

(B) Micrographs showing the mitotic spindle in cells 1 hr after release at 37°C from S phase. Wild-type (VG982–6A) and *mcd1* (VG985–7C) cells were synchronously released at 37°C from S phase arrest, and after 1 hr cells were processed for immunofluorescence. Chromosomal DNA (top) and microtubules (bottom).

(C) Quantitation of cell and DNA morphologies after release at 37°C from S phase. Strains in B were scored for cell and DNA morphologies in S phase–arrested cells at 37°C [S (HU)] and in cells 2 hr after release (2 hr after S). Data from 200–300 cells from two independent experiments were scored to generate error bars.

mcd1–1 cells were further characterized using synchronous populations obtained after release at 37°C from S phase arrest. While arrested in S phase, wild-type and *mcd1* cells had a 1C DNA content, large bud, undivided DNA mass, and short spindle (Figure 2C). After release (1 hr), most wild-type cells completed both DNA replication and chromosome segregation since cells had a 2C DNA content, elongated spindle, and segregated DNA masses (Figure 2B). In contrast, most *mcd1* cells had a stretched nuclear DNA mass and partially elongated spindle indicative of a chromosome segregation defect (Figure 2B). By 2 hr, 50% of wild-type cells exited mitosis (unbudded and small budded cells), compared to only 20% for *mcd1* cells (Figure 2C). The majority of *mcd1* cells remained in mitosis with cell and DNA morphologies similar to that at 1 hr (Figure 2C). Similar results were obtained when Mcd1p was depleted

in strains in which the sole source of *MCD1* was under control of an inducible promoter (data not shown). These data suggest that *mcd1* inactivation causes a mitotic defect that disrupts chromosome segregation and delays, but does not prevent, exit from mitosis. Finally, *mcd1* cells exhibited a 10 min delay in early S phase, indicating a requirement for Mcd1p at this time (data not shown).

Mcd1p Is Required for Sister Chromatid Cohesion

To establish that *mcd1–1* cells are defective in sister chromatid cohesion, synchronized populations of wild-type and *mcd1* haploid cells were arrested in mid-M phase by either of two regimens and subjected to FISH. For regimen 1, cells were at the nonpermissive temperature for the *mcd1–1* mutation through S and mid-M phases, which is the time sister chromatid cohesion is both established and maintained (Experimental Procedures). Cells were processed for FISH using either a chromosome XVI centromere-proximal (CEN-proximal) or distal probe to assay cohesion at different sites along chromosome XVI.

Consistent with our previous studies, in mid-M phase most wild-type cells had one FISH signal per nuclear DNA mass, demonstrating that sister chromatids were paired (Figures 3A and 3B). In a small number of DNA masses, two FISH signals were detected due to either a low level of precocious sister chromatid dissociation or spurious background (Figure 3B). In contrast, most *mcd1* cells in mid-M phase at 37°C had two FISH signals per DNA mass, indicating that sister chromatids had precociously dissociated (Figures 3A and 3B). The cohesion defect is not restricted to nocodazole arrested cells since inactivation of Mcd1p also caused precocious sister chromatid dissociation in cells arrested in G2/M by a mutation in an anaphase promoting complex (APC) subunit (Cdc16p). For example, 60% of *mcd1 cdc16* cells exhibited precocious sister separation at CEN-proximal and distal loci compared to only 15% in *cdc16* cells. The second FISH signal in mid-M phase *mcd1* cells was not due to preexisting aneuploidy since in G1 phase, most cells had only one FISH signal (Figure 3B). The few cells that had two FISH signals in G1 in wild-type and *mcd1* cells were likely due to spurious background. Similar results were obtained using probes from CEN-proximal regions of chromosomes I and IV and a more CEN-distal chromosome XVI region (data not shown). Thus, Mcd1p is required for sister chromatid cohesion at CEN-proximal and distal chromosomal regions in yeast cells.

The previous regimen can not distinguish between defects in establishment or maintenance of cohesion. To assay for maintenance of cohesion, *mcd1–1* and wild-type cells were allowed to establish cohesion at permissive temperature before *mcd1–1* function was inactivated (Experimental Procedures, regimen 2). As expected, mid-M wild-type cells at 23°C and 37°C had one FISH signal per DNA mass (Figure 3C). Most mid-M *mcd1* cells had one FISH signal at 23°C but two signals upon shift to 37°C. Similar results were obtained using probes from chromosomes I, IV, and XVI (data not shown). These results demonstrate that Mcd1p is required for maintenance of sister chromatid cohesion.

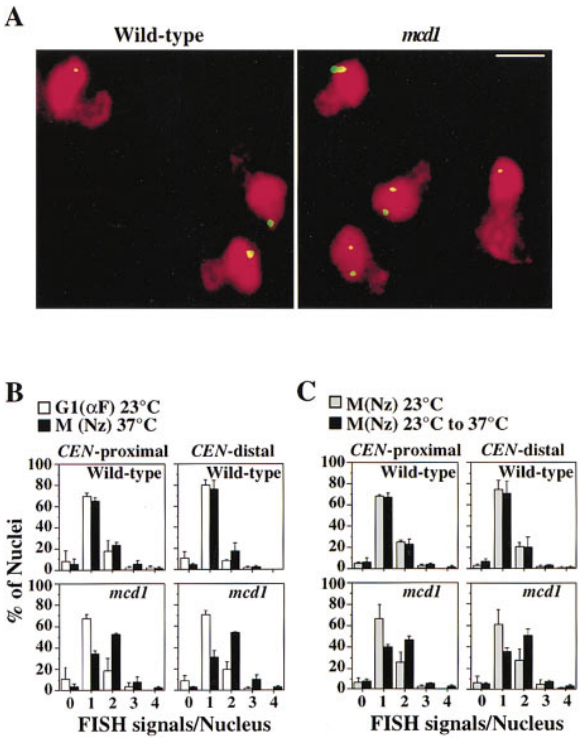

Figure 3. Analysis of Sister Chromatid Cohesion in Mid-M Phase Wild-Type and *mcd1–1* Cells

(A) Micrographs of mid-M cells subjected to FISH using a chromosome XVI CEN-distal probe. Wild-type (VG906–1A) and *mcd1* (VG955–7D) cells arrested in G1 phase [G1 (αF) 23°C] were released from G1 and arrested in mid-M phase [M (Nz) 37°C]. Mid-M cells were processed for FISH. Chromosomal DNA (red) and hybridized probe (green). Bar is 5 μm. (B) Quantitation of FISH for G1 and mid-M cells. Cells described in (A) were hybridized with either a chromosome XVI CEN-proximal (23 kb from CEN16) or distal (295 kb from CEN16) probe. The number of FISH signals in each DNA mass was determined and plotted as a percentage of total nuclei. For each probe, 200 nuclei from two independent experiments were scored to generate data and error bars. (C) Quantitation of FISH for cells arrested in mid-M phase at 23°C then incubated at 37°C. Strains described in (A) were arrested in G1 phase at 23°C, released from G1, and arrested in mid-M phase at 23°C [M (Nz) 23°C] then transferred to 37°C [M (Nz) 23°C to 37°C]. For each probe, at least three hundred nuclei from three independent experiments were scored and data plotted as described in (B).

Mcd1p Is Required for Chromosome Condensation

The stretched DNA mass in *mcd1* mutants at 37°C is reminiscent of yeast mutants defective in condensation (Strunnikov et al., 1995). To test the role of Mcd1p in condensation, we used FISH to examine chromosome condensation at the rDNA locus, a 500 kb block of repetitive DNA (Guacci et al., 1994). We had shown that in G1 phase cells, an amorphous rDNA FISH signal characteristic of a decondensed chromosome is detected, while in mid-M phase haploid cells a single line-like FISH signal characteristic of condensed and paired sister chromatids is seen (Guacci et al., 1994). As expected, wild-type cells arrested in mid-M using regimen 2 had a single line-like FISH signal in 85% of the DNA masses (Figure 4A). In contrast, 74% of DNA masses from similarly treated *mcd1* cells had an amorphous FISH signal (Figure 4A). An amorphous FISH signal is not expected for

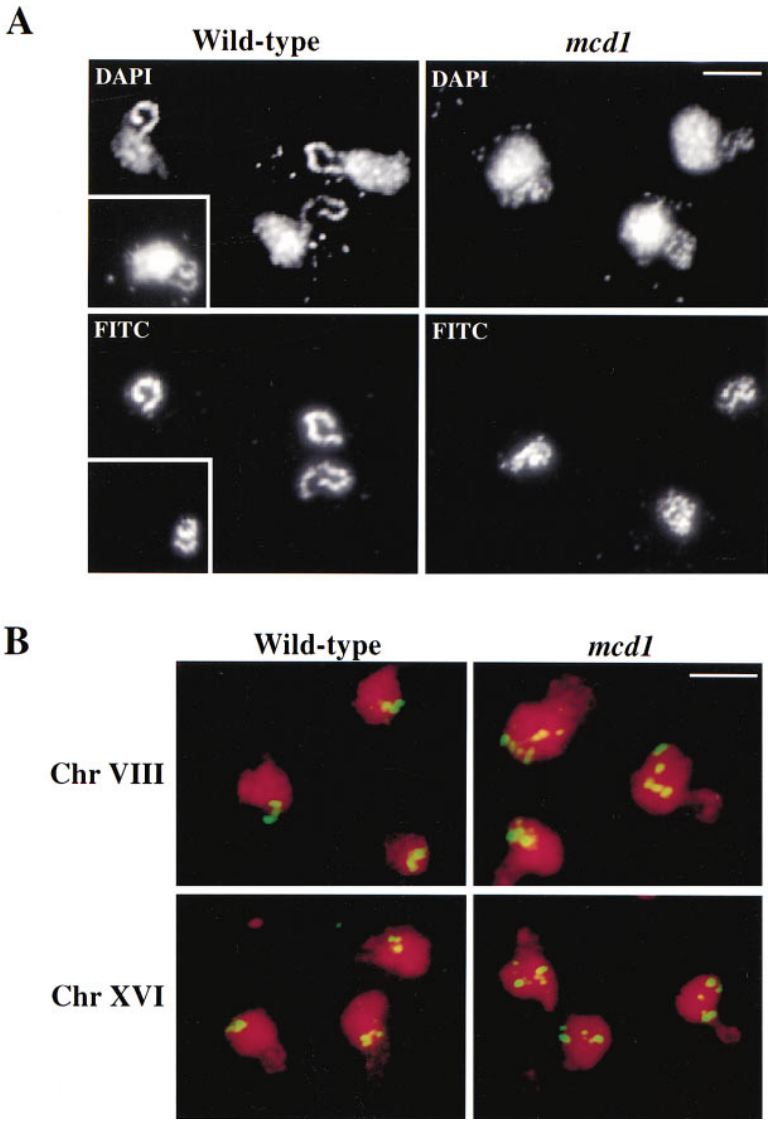

Figure 4. Analysis of Chromosome Condensation in Wild-Type and *mcd1–1* Cells in Mid-M Phase

(A) Micrographs showing FISH of the rDNA. Wild-type (VG906–1A) and *mcd1* (VG955–7D) cells were synchronously arrested in mid-M at 23°C then incubated 37°C while arrested as described in the legend to Figure 3C. Mid-M phase cells at 37°C were subjected to FISH using rDNA as probe. Chromosomal DNA (DAP) and hybridized probe (FIT) are shown. Bar is 5 μm. (B) Micrographs showing FISH of regions of chromosomes VIII and XVI in mid-M cells. Strains in (A) were synchronously arrested in mid-M at 37°C then subjected to FISH using a mixture of chromosome VIII or chromosome XVI probes. Chromosomal DNA (red) and hybridized probes (green) are shown. Bar is 5 μm.

separation of sister chromatid, since in wild-type cells precociously separated sisters are seen as two line-like signals (Figure 4A, inset). Furthermore, the rDNA is line-like after sister chromatids have separated and segregated in anaphase nuclei cycling wild-type cells (Guacci et al., 1994). Thus, *mcd1–1* cells in mid-M phase at 37°C have an aberrant rDNA morphology indicative of a defect in rDNA condensation.

To monitor chromosome condensation at unique chromosomal regions, synchronized populations of wild-type and *mcd1* haploid cells arrested in mid-M phase at 37°C (regimen 1) were subjected to FISH using a mixture of four chromosome XVI probes or six chromosome VIII probes. The number and spacing of FISH signals from the mixture of chromosome XVI or VIII probes provides a qualitative measure of chromosome condensation. For example, when haploid cells are arrested in G1 phase and hybridized with chromosome XVI probes, up to four, often widely spaced, FISH signals are detected per DNA mass whereas in mid-M phase, one or two closely associated FISH signals are detected (Guacci et al., 1994). This change from dispersed in G1 phase to clustered in mid-M phase is characteristic of the change from a decondensed chromosome to a condensed chromosome with paired sisters.

As expected, in wild-type cells arrested in mid-M phase at 37°C and hybridized with either the chromosome XVI or VIII probe mixtures, a few closely associated FISH signals were detected in most DNA masses as expected for condensed and paired sister chromatids (Figure 4B). In contrast, in *mcd1* cells hybridized with either the chromosome XVI or VIII probe mixtures, many, often widely spaced FISH signals were detected in many DNA masses (Figure 4B). Some of the increased numbers of FISH signals are expected due to sister chromatid dissociation. However, if sister chromatids remained condensed, there should be two tight clusters of FISH signals, one from each separated and condensed sister chromatid. Instead, the dispersed FISH signals are reminiscent of decondensed chromosomes (Guacci et al., 1994). Taken together, the results from FISH using rDNA, chromosome VIII, and chromosome XVI probes indicate

that the *mcd1–1* mutant exhibits defects in chromosome condensation as well as sister chromatid cohesion.

Mcd1p Is Nuclear and Its Levels Are Cell Cycle Regulated

To determine if the in vivo localization of Mcd1p was consistent with its proposed role as a chromosomal structural protein, cells were processed for both Western blot analysis and indirect immunofluorescence using anti-Mcd1p antibodies. Initially, we examined a strain in which Mcd1p was overexpressed (Figure 5A). Many cells had prominent punctate nuclear staining, with the exception of cells undergoing anaphase, where Mcd1p was dispersed evenly throughout the cell, suggesting possible redistribution (Figure 5B). When expressed from its endogenous promoter in wild-type cells, Mcd1p staining was highly variable but nuclear when detected (data not shown). To examine whether this variability was cell–cycle dependent, wild-type cells were arrested in G1, S, or mid-M phase and processed for Western blotting and indirect immunofluorescence. Mcd1p was barely detectable in G1 phase, at high levels in S phase, and at lower levels in mid-M phase with a punctate nuclear localization in S and mid-M phases (Figures 5C and 5D). Similarly, the S. pombe Rad21 protein also localized to the nucleus (Birkenbihl and Subramani, 1995). The nuclear localization of Mcd1p during S phase and mitosis is consistent with its proposed role in chromosome structure.

To monitor the dynamics of cell-cycle dependent changes in Mcd1p levels, a synchronous population of wild-type cells was examined. Aliquots of cells were taken in G1 phase, as well as every 20 min after release from G1, then processed to monitor Mcd1p levels and scored for DNA content to assess cell cycle position. Mcd1p levels were barely detectable in G1 phase cells (T = 0), reached a peak in S phase (T = 40) for many cells, and decreased in G2/M phases (T = 60) (Figure 6A). To more precisely determine the time when Mcd1p levels decline, the relative levels of Mcd1p and Pds1p were compared. The decrease in Mcd1p levels occurred 20 min prior to the decrease in Pds1p levels (Figure 6B, arrows). Since Pds1p degradation is required for the metaphase-to-anaphase transition, the decrease in Mcd1p levels occurred before this cell cycle progression landmark (Cohen-Fix et al., 1996). Finally, Mcd1p levels were examined by Western at discrete cell cycle stages using *cdc* mutants or wild-type cells treated with α factor, HU, or Nz. Mcd1p was barely detectable in G1 (*cdc28–4*, α factor), peaked in early S (HU), and decreased to a lower but steady level in late S (*cdc9*), G2 (*cdc28–1N*), G2/M (*cdc13* and *cdc16*), mid-M (*cdc20*, *cdc23*, and Nz), and telophase (*cdc14* and *cdc15*) cells (data not shown). Taken together, these data show that Mcd1p levels peak in early S phase, are reduced by late S to a lower level that remains constant through telophase and decreases to nearly undetectable levels by G1.

Insights into the cell cycle–dependent changes in Mcd1p levels came from examination of *MCD1* mRNA in synchronized cycling cells and Mcd1p levels in *cdc* mutants. *MCD1* mRNA levels peak in early S phase then decrease 9-fold 20 min prior to the Mcd1p decrease (Figure 6B and 6C). This pattern of mRNA regulation is likely due to two MluI boxes in the *MCD1* promoter region (data not shown; McIntosh et al., 1991). The decrease in mRNA coupled with possible PEST-mediated degradation is sufficient to account for the decrease in Mcd1p levels in late S. Mcd1p levels dropped 20 min prior to Pds1p degradation, a known landmark of anaphase initiation and the earliest known target of the anaphase promoting complex, APC (Figure 6B). Moreover, the decrease in Mcd1p levels observed in late S/G2 was not affected by mutations in APC components (*cdc16* and *cdc23*) (data not shown). Thus, the change in Mcd1p levels in late S/G2 is independent of APC, although it may play a role in the subsequent decrease in Mcd1p levels in G1.

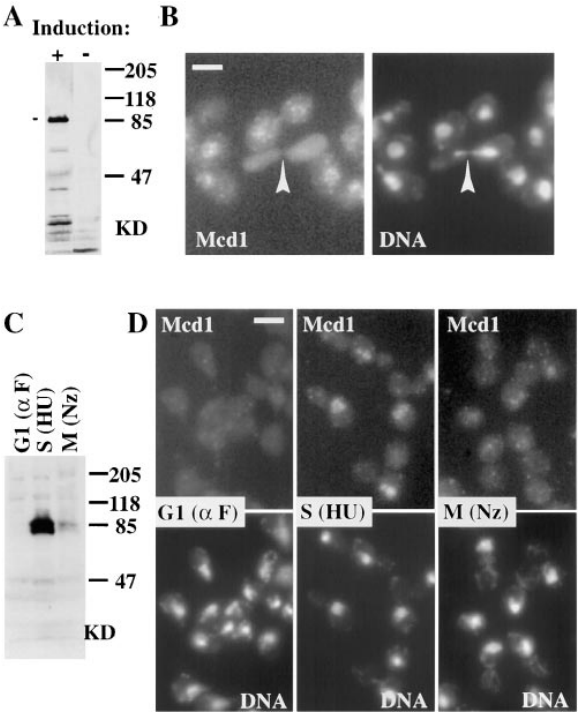

Figure 5. In Vivo Localization of Mcd1p

(A) Specificity of affinity-purified anti-Mcd1 Ab. Strain YPH499b/pAS339 was grown under conditions to induce *MCD1* overexpression (Induction +) or to repress *MCD1* expression (Induction −). Cells were processed for Western blot and probed with anti-Mcd1p Ab. The protein equivalent of 10^7 cells was loaded per lane. (B) Redistribution of Mcd1p in mitosis. Mcd1p was overexpressed in asynchronously growing cells (YPH499b/pAS339) then subjected to indirect immunofluorescence using anti-Mcd1p Ab. Arrow indicates an anaphase cell. (C) Cell cycle–dependent changes in Mcd1p levels by Western blot. Wild-type strain YPH499b was arrested in either G1 [G1 (αF)], S [S (HU)] or mid-M [M (Nz)] phase and Mcd1p levels monitored using anti-Mcd1p Ab. (D) Cell cycle–dependent changes in Mcd1p levels in vivo. Cells in (C) were processed for indirect immunofluorescence using anti-Mcd1p Ab.
Bar is 5 μm.

Discussion

We identified the *MCD1* gene and demonstrated that it encodes a conserved protein necessary for both sister

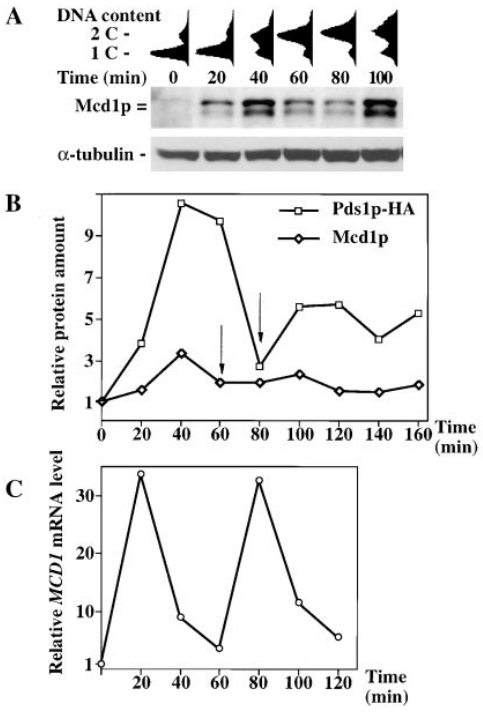

Figure 6. Cell Cycle–Dependent Expression of Mcd1p

(A) Mcd1p levels in synchronized cycling cells. Wild-type strain YPH499b was arrested in G1 phase (T = 0) then released from G1 and aliquots taken every 20 min for Western analysis and flow cytometry. Mcd1p was detected using anti-Mcd1p Ab whereas protein loading was shown by monitoring α tubulin levels using YOL1/34 Ab. (B) Relative levels of Pds1p and Mcd1p in synchronized cycling cells. Strain OCF1522 was grown as described in (A). For each time point, Mcd1p and Pds1p levels were determined by Western analysis using anti-Mcd1p Ab and anti-HA Ab, respectively, then normalized to tubulin levels as detected in (A). Relative protein amounts were calculated by comparing the normalized value for G1 cells (T = 0), arbitrarily set at 1, to that at each time point and the results plotted. Arrows indicate the distinct curve minima for Mcd1p and Pds1p-HA levels. (C) *MCD1* mRNA levels are cell cycle regulated. Cells in B were subjected to Northern analysis using *MCD1* probe to monitor mRNA levels. mRNA levels were normalized to *ACT1* mRNA levels.

chromatid cohesion and condensation in budding yeast. Mcd1p acts throughout the genome, as it is required for cohesion at *CEN*-proximal and distal (arm) sequences as well as for condensation at three different chromosomal regions, including both unique and repetitive DNA. A cohesion function for Mcd1p (Scc1p) has also been demonstrated by independent methods (Michaelis et al., 1997). We show that Mcd1p and Smc1p interact genetically and physically. Based on these interactions and previous observations that other SMC proteins are chromosomal, we suggest that Mcd1p functions in cohesion and condensation as a component of mitotic chromosomes. Our functional assays place Mcd1p as a chromosomal protein. Consistent with this conclusion, Mcd1p appears to localize to chromosomes in an Smc1p-dependent manner (Michaelis et al., 1997). Finally, we show that both the Mcd1p and *MCD1* mRNA levels are cell cycle–regulated such that Mcd1p persists from the time cohesion is first established (S phase) until the time when chromosomes decondense (telophase), consistent with its role in chromosome cohesion and condensation.

Mcd1p, a Novel Chromosomal Protein Required for Mitotic Sister Chromatid Cohesion

The identification and characterization of Mcd1p has provided several new insights into the process of sister chromatid cohesion. Mcd1p is a novel conserved chromosomal protein shown to be required for cohesion during mitosis. This conservation implicates a common mechanism for mitotic sister chromatid cohesion among eukaryotes. The requirement of Mcd1p for cohesion at both centromeric and arm sequences indicates that these regions share a common underlying mechanism of cohesion. In many eukaryotes, a differential dissolution of cohesion at centromeres and arm regions has been observed in meiosis and mitosis. It will be interesting to determine how the common mechanism of cohesion is modulated to give region-specific responses. A candidate for a region-specific cohesion factor is the Drosophila MEI-S332 protein, which is essential for meiotic centromere cohesion (Kerrebrock et al., 1995).

Both APC-dependent proteolysis and protein phosphorylation have been implicated in the dissolution of sister chromatid cohesion at the metaphase-to-anaphase transition (Ohkura et al., 1989; Holloway et al., 1993; Cohen-Fix et al., 1996; Minshull et al., 1996; Yamamoto et al., 1996b). While the levels of Mcd1p are regulated during the cell cycle, the decrease in Mcd1p levels occurred prior to the metaphase-to-anaphase transition and was APC independent. Furthermore, significant amounts of Mcd1p persisted to telophase. Therefore, the degradation of Mcd1p, a chromosomal component of cohesion, is unlikely to play a role in the dissolution of cohesion. However, Mcd1p activity may be regulated by modification. Indeed, the Rad21 protein, the S. pombe homolog of Mcd1p, has multiple cell cycle–regulated forms due to phosphorylation, and Mcd1p migrates as a doublet, raising the possibility of its phosphorylation (Birkenbihl and Subramani, 1995; this study). Further analysis of Mcd1p and the identification of other chromosomal components of cohesion will be necessary to assess the relative contributions of proteolysis, phosphorylation, or other modifications to the dissolution of cohesion.

Mcd1p, a Global Chromosome Condensation Factor

Mcd1p is a novel protein in budding yeast whose function has been shown to be important for condensation of both unique and repetitive sequences (this study). Other proteins like Smc2p, Trf4p, and Top1p have been shown to be important for condensation at a subset of chromosomal loci (Strunnikov et al., 1995; Castano et al., 1996). We suggest that Mcd1p is a key component of the general condensation machinery that may be acted upon by region-specific factors.

Mcd1p is a new conserved SMC-associated protein in addition to Trf4p, Top1p, and the non-SMC condensin

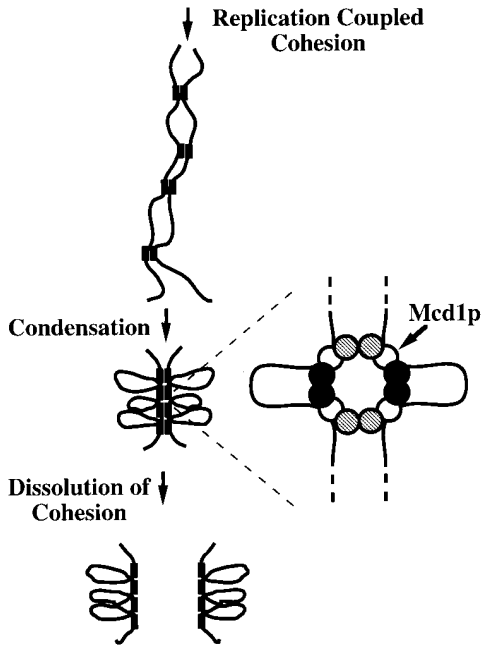

Figure 7. Model for Mitotic Chromosome Structure

Sister chromatid cohesion is established during S phase by assembly of chromosomal core components (open circles) like Mcd1p and cohesion-specific factors (hatched circles) at DNA cohesion sites (rectangles). Condensation is achieved by coalescence of the cohesion complexes by condensation-specific factors (closed circles). It should be noted that the model does not account for additional putative folds that lead to higher order compaction of the loops and axis (reviewed in Koshland and Strunnikov, 1996). The model does not demand DNA replication for condensation, assuming that these factors can associate with cohesion sites of unreplicated chromosomes. At the metaphase-to-anaphase transition, cohesion-specific factors are either degraded or modified to allow sister chromatid separation. At telophase, condensation factors are inactivated to allow decondensation (not shown).

components (Strunnikov et al., 1993, 1995; Castano et al., 1996; Hirano et al., 1997; this study). The addition of Mcd1p to a growing list of condensation factors indicates that condensation is biochemically complex, as might be expected for a highly determined process (reviewed in Koshland and Strunnikov, 1996). Finally, the conservation of Mcd1p further emphasizes that the mechanism of condensation is conserved.

Mcd1p Links Sister Chromatid Cohesion and Condensation

Perhaps the most profound insight from this study is that Mcd1p functions in both sister chromatid cohesion and chromosome condensation, hence linking these two processes previously thought to be independent. A model to explain this link must incorporate the facts that cohesion can exist without condensation (during S and G2 phases), cohesion and condensation must coexist (prophase through metaphase), and condensation can exist without cohesion (anaphase to telophase). We propose a simple model that explains these observations and the link between cohesion and condensation (Figure 7). Sites of cohesion (rectangles) are distributed along the length of the chromosome. Soon after replication, the sister chromatids are held together at these sites. To condense the chromosome, cohesion sites are brought together, and consequently, the chromosomal regions between these sites are looped out (Figure 7). Proteins that mediate cohesion (hatched circles) and condensation (closed circles) are distinct but interact with common chromosomal core components (open circles) such as Mcd1p. These processes can be regulated independently by controlling the association of factors involved exclusively in either cohesion or condensation.

This model explains many observations about the structure of mitotic chromosomes. First, loops have been observed both in partially denatured mitotic chromosomes and in lampbrush chromosomes (Adolph et al., 1977; Paulson and Laemmli, 1977). Second, condensed sister chromatids exhibit mirror symmetry (Boy de la Tour and Laemmli, 1988; Baumgartner et al., 1991). It was previously thought that condensation occurred by a mirror symmetric mechanism which allowed sister chromatid cohesion to persist (Boy de la Tour and Laemmli, 1988). In contrast, our model suggests that cohesion is the cause of mirror symmetric condensation. Third, defects in some mitotic structural proteins give rise specifically to condensation defects. Mutants in *smc2* are defective in condensation but not cohesion (Strunnikov et al., 1995; E. Hogan and D. K., unpublished data). Candidate mutants defective for cohesion but not condensation of repetitive DNA also exist (V. G. and D. K., unpublished data).

Our model also predicts that a higher density of cohesion sites leads to smaller loops and less condensation, which can explain some differences between mammalian and budding yeast chromosome structure. FISH analysis of sister chromatids prior to and after condensation reveals two closely juxtaposed FISH signals in mammalian cells but only one signal in yeast (Selig et al., 1992; Guacci et al., 1993, 1994). We suggest that this difference can be explained by a higher density of cohesion sites in budding yeast leading to the observed 2.5- to 5- fold reduction in compaction compared to mammalian chromosomes (Lawrence et al., 1988; Guacci et al., 1994). Finally, we have candidates for cohesion sites in budding yeast (P. Megee and D. K., unpublished data), and manipulation of these sites should enable a direct test of their role in condensation.

In addition to the link between cohesion and condensation of mitotic chromosomes, other potential links are revealed by the properties of Mcd1p and the *mcd1* mutant. Mcd1p levels change during S phase and the *mcd1* mutant exhibits a G1/S delay (this study; V. G. and D. K., unpublished data). Since cohesion and condensation are thought to initiate in S phase (Rao and Adlakha, 1984; Selig et al., 1992; Guacci et al., 1994; Koshland and Strunnikov, 1996), this delay indicates that cells may use checkpoints to assure the proper assembly of chromosomes so that they are competent for subsequent segregation in mitosis. Consistent with this idea, G1/S delay has been observed in kinetochore mutants (Connelly and Hieter, 1996; Saitoh et al., 1997). Furthermore, the fact that both the *mcd1* mutant and the S. pombe *rad21* mutant are radiation sensitive (Birkenbihl and Subramani, 1992; V. G. and D. K., unpublished data) suggests a possible link between chromosome structure and the fidelity or efficiency of DNA damage repair.

Table 1. Yeast Strains Used in This Study

Strain	Genotype
VG925–2A	Matα *mcd1–1 trp1 ura1 gal1*
VG995	Matα *mcd1::URA3 leu2 ura3 gal1*
	Mat**a** *trp1 ura3 gal1*
VG906–1A	Mat**a** *trp1 leu2 bar1 gal1*
VG955–7D	Mat**a** *mcd1–1 trp1 leu2 bar1 gal1*
VG982–6A	Mat**a** *trp1 ura3 bar1 gal1*
VG985–7C	Mat**a** *mcd1–1 trp1 ura3 bar1 gal1*
VG1309–7B	Mat**a** *cdc 16–1 trp1 ura3 bar1 gal1*
VG1301–8D	Mat**a** *mcd1–1 cdc16–1 trp1 ura3 can1 bar1 gal1*
CP16–2	Mat**a** *pds1–2 cdc16–1 his7 hom3 can1 bar1 gal1*
VG1354–1B	Mat**a** *cdc13–1 trp1 ura3 leu2 bar1 gal1*
YPH4996	Mat**a** *trp1 ura3 leu2 bar1 ade2 his3 lys2*
OCF1522	Mat**a** *PDS1–HA:URA3 ade2 his3 trp1 bar1*
AS321	Matα *mcd1–Δ3::HIS3 ura3 ade2 leu2 lys2 his3*
	Mat**a** *trp1 ura3 ade2 leu2 lys2 his3*
YPH499b/pAS339	Mat**a** *mcd1::pGAL1–MCD1–URA3 ade2 his3 leu2 lys2 trp1 ura3–52 bar1*
3aAS273	Mat**a** *smc1–2:LEU2 ade2 his3 leu2 lys2 ura3*
BS334/pAS339	Matα *reg1–501 pep4–3 prb1–1122 leu2 ura3 gal1 mcd::1pGAL–MCD1–URA3*

Experimental Procedures

Reagents and Media

Reagents were described (Yamamoto et al., 1996b; Guacci et al., 1997). Benomyl was a gift from Dupont. Standard media were made as described (Rose et al., 1990). YPRG and YPDG liquid contained 1% yeast extract, 2% peptone, 2% galactose, and either 2% raffinose or 2% dextrose, respectively. Yeast transformation, genetic manipulation, and plasmid isolation were described (Rose et al., 1990; Robzyk and Kassir, 1992). Yeast strains are listed in Table 1.

Cloning and Mapping of *MCD1*

MCD1 was cloned in two ways. First, the *mcd1–1* mutant was backcrossed and the Ts^- and Mcl^- phenotypes each segregated 2+:2− and cosegregated in all 27 tetrads. The Ts^- phenotype was tightly linked to CEN4 (no recombination in 79 tetrads). Strain VG925–2A (*mcd1*) was transformed with a CEN vector-based yeast genomic library (provided by P. Hieter). Transformants (20,000) were replica plated to YPD at 37°C, and the four Ts^+ transformants obtained had the same plasmid clone. A 2.8 kb XhoI/BglII fragment was inserted between the XhoI and BamHI sites of plasmid pRS316 (Sikorski and Hieter, 1989), and the resulting plasmid, pVG164, complemented the *mcd1* Ts^- and Mcl^- phenotypes. Second, strain 3aAS273 (*smc1–2*) was transformed with a 2μ vector-based yeast genomic library (provided by J. Boeke). Several transformants complemented the *smc1–2* Ts^- phenotype and contained the same plasmid. A 2.8 kb XhoI/BglII fragment was inserted between the XhoI and BamHI sites of plasmid pRS426 (Sikorski and Hieter, 1989) and the resulting plasmid, pAS271/3, complemented the *smc1–2* Ts^- phenotype. Restriction mapping revealed that the 2.8 kb XhoI/BglII fragments from pVG164 and pAS271/3 were identical. The insert was sequenced (gene bank sequence U23759) and, when compared to the yeast genome sequence, mapped 3.3 kb from *CEN4*, consistent with the meiotic mapping data for *mcd1–1* (see above).

Diploid strain (AS321) was made heterozygous for a complete deletion of *MCD1* using a PCR deletion method (Baudin et al., 1993). *MCD1* was also placed under control of an inducible *GAL1* promoter (*pGAL:MCD1*) by inserting a BclI-HincII *MCD1* fragment generated by PCR into plasmid pAS89 (Strunnikov et al., 1993) to form plasmid pAS339. This plasmid was linearized with BstEII, transformed into yeast to integrate *pGAL:MCD1* at the *MCD1* locus and to truncate the endogenous *MCD1* gene to form strains YPH499b/pAS339 and BS334/pAS339. Integrations were confirmed by Southern blot.

Yeast Cell Culture Conditions

Mid-log cultures (YPD, 23°C) were treated as follows:

Arrest at G1, S, or Mid-M Phase

α factor (10^{-8}M), HU (100 mM), or Nz (15 μg/ml′′) was added and cells incubated 3 hr at 23°C (or 30°C when appropriate) to arrest in G1, S, or mid-M phase, respectively. When required, cultures were transferred to 37°C for 2 hr while arrested. Percent cell viability was determined as described (Yamamoto et al., 1996b).

Synchronous Populations of Mid-M Phase–Arrested Cells

G1 arrested cells (see above) were washed with either: YPD + Nz + pronase E (0.1 mg/ml) at 37°C, then incubated 2 hr at 37°C in YPD + Nz + pronase to arrest cells in mid-M (Regimen 1); or YPD + Nz + pronase at 23°C, then incubated 2 hr at 23°C in YPD + Nz + pronase to arrest cells in mid-M and incubated 1 hr at 37°C (Regimen 2).

Synchronous Populations of Cycling Cells: Release from G1 Phase

G1 arrested cells at 30°C were released from G1 by washing and incubation in YPD + pronase at 30°C.

Release from S Phase

S phase arrested cells were shifted to 37°C for 1 hr while arrested. Cells were released from S by washing and incubation at 37°C in YPD.

Overexpression of Mcd1p in Cells

Strains YPH499b/pAS339 and BS334/pAS339 were grown in YPRG and YPDG, respectively, at 30°C to induce *MCD1* overexpression from *pGAL:MCD1*.

Microscopy and Flow Cytometry

Flow cytometry was as described (Yamamoto et al., 1996a). Indirect immunofluorescence was as described (Kilmartin and Adams, 1984). Mcd1p was detected by affinity-purified anti-Mcd1p antibodies (Ab) diluted 1:1000 as described for microtubules, except cells were fixed 1 hr at 23°C. Images were collected using a Zeiss epifluorescence microscope and recorded digitally using a Princeton CCD camera with Signal Analytics processing software, which allowed image superimposition.

Fluorescence In Situ Hybridization (FISH)

FISH was performed as described (Guacci et al., 1994) with the following minor changes. Spheroplasted cells were resuspended in 0.5 M sorbitol, 0.5% Triton X-100, added to slides, and incubated 10 min. Sorbitol/Triton was replaced with 1% SDS and treated as described. Probes were labeled with digoxigenin as described (Guacci et al., 1994). Probes from chromosomes I, IV, XII, and XVI were described (Guacci et al., 1994, 1997).

Six probes from the right arm of chromosome VIII were made from cosmids containing a yeast DNA insert. The insert sizes were 26 kb to 42 kb and spanned a 266 kb region that is 137 kb from *CEN8*. Cosmids were purchased from the American Tissue Culture Collection.

Antibodies and Protein Analysis

The NheI-Asp718 fragment from pAS271/3 was cloned into corresponding sites of pRSETa (Invitrogen) to express a 29 kDa Mcd1p

fragment in bacteria. The resultant plasmid was transformed into the BL21(DE3) pLysS strain of E. coli (Novagen), and the Mcd1p fragment purified by IMAC (ProBond, Invitrogen) then PAGE and injected into NZW rabbits (Covance). Bleeds were affinity purified on CNBr sepharose columns (Pharmacia) with the coupled antigen. Anti-Mcd1p Ab was used in 1:2000 dilution for Western blots while 12CA5 monoclonal Ab (Boehringer) and YOL1/34 Ab (Harlan) were used to monitor Pds1p-HA and α-tubulin, respectively. For immunoprecipitations, monoclonal anti-T7 tag Ab (Novagen), rabbit polyclonal anti-Smc1p Ab and sera depleted of anti-Smc1p Ab were as described (Strunnikov et al., 1993, 1995).

For analytical purposes, cells were washed with 0.15 M NaCl with protease inhibitors, and total yeast protein extracted by breaking cells with glass beads in 2% SDS with Complete protease inhibitors (Boehringer) using a Multi-Tube Vortexer (VWR). Protein concentration in boiled samples was assessed using Pierce protein assay and SpectraMax340 microplate reader (Molecular Devices). Quantification of Western blot ECL signal was performed using a Molecular Dynamics densitometer.

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References

Adolph, K.W., Cheng, S.M., and Laemmli, U.K. (1977). Role of nonhistone proteins in metaphase chromosome structure. Cell *12*, 805.

Baudin, A., Ozier-Kalogeropoulos, O., Denouel, A., Lacroute, F., and Cullin, C. (1993). A simple and efficient method for direct gene deletion in Saccharomyces cerevisiae. Nucleic Acids Res. *21*, 3329–3330.

Baumgartner, M., Dutrillaux, B., Lemieux, N., Lillienbaum, A., Paulin, D., and Viegas-Péquignot, E. (1991). Genes occupy a fixed and symmetrical position on sister chromatids. Cell *64*, 761–766.

Birkenbihl, R.P., and Subramani, S. (1992). Cloning and characterization of rad21, an essential gene of Schizosaccharomyces pombe involved in DNA double-strand-break repair. Nucleic Acids Res. *20*, 6605–6611.

Birkenbihl, R.P., and Subramani, S. (1995). The rad21 gene product of Schizosaccharomyces pombe is a nuclear, cell cycle–regulated phosphoprotein. J. Biol. Chem. *270*, 7703–7711.

Boy de la Tour, E., and Laemmli, U.K. (1988). The metaphase scaffold is helically folded: sister chromatids have predominantly opposite helical handedness. Cell *55*, 937–944.

Castano, I., Brzoska, P., Sadoff, B., Chen, H., and Christman, M. (1996). Mitotic chromosome condensation in the rDNA requires *TRF4* and DNA topoisomerase I in Saccharomyces cerevisiae. Genes Dev. *10*, 2564–2576.

Chuang, P.T., Albertson, D.G., and Meyer, B.J. (1994). DPY-27: a chromosome condensation protein homolog that regulates C. elegans dosage compensation through association with the X chromosome. Cell *79*, 459–474.

Cohen-Fix, O., Peters, J., Kirschner, M., and Koshland, D. (1996). Anaphase initiation in Saccharomyces cerevisiae is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. Genes Dev. *10*, 3081–3093.

Connelly, C., and Hieter, P. (1996). Budding yeast *SKP1* encodes an evolutionarily conserved kinetochore protein required for cell cycle progression. Cell *86*, 275–285.

Cooke, C.A., Heck, M.M.S., and Earnshaw, W.C. (1987). The inner centromere protein (INCENP) antigens: movement from inner centomere to midbody during mitosis. J. Cell Biol. *105*, 2053–2067.

Coux, O., Tanaka, K., and Goldberg, A.L. (1996). Structure and functions of the 20S and 26S proteasomes. Annu. Rev. Biochem. *65*, 801–847.

Funabiki, H., Yamano, H., Kumada, K., Nagao, K., Hunt, T., and Yanagida, M. (1996). Cut2 proteolysis required for sister chromatid separation in fission yeast. Nature *381*, 438–441.

Grunstein, M. (1997). Molecular model for telomeric heterochromatin in yeast. Curr. Opin. Cell Biol. *9*, 383–387.

Guacci, V., Yamamoto, A., Strunnikov, A., Kingsbury, J., Hogan, E., Meluh, P., and Koshland, D. (1993). Structure and function of chromosomes in mitosis of budding yeast. Cold Spring Harb. Symp. Quant. Biol. *58*, 677–685.

Guacci, V., Hogan, E., and Koshland, D. (1994). Chromosome condensation and sister chromatid pairing in budding yeast. J. Cell Biol. *125*, 517–530.

Guacci, V., Hogan, E., and Koshland, D. (1997). Centromere position in budding yeast: evidence for anaphase A. Mol. Biol. Cell *8*, 957–972.

Hirano, T., and Mitchison, T.J. (1994). A heterodimeric coiled-coil protein required for mitotic chromosome condensation in vitro. Cell *79*, 449–458.

Hirano, T., Kobayashi, R., and Hirano, M. (1997). Condensins, chromosome condensation protein complexes containing XCAP-C, XCAP-E, and a Xenopus homolog of the Drosophila Barren protein. Cell *89*, 511–521.

Holloway, S.L., Glotzer, M., King, R.W., and Murray, A.W. (1993). Anaphase is initiated by proteolysis rather than by the inactivation of maturation-promoting factor. Cell *73*, 1393–1402.

Hoyt, M.A., Totis, L., and Roberts, B.T. (1991). S. cerevisiae genes required for cell cycle arrest in response to loss of microtubule function. Cell *66*, 507–517.

Jessberger, R., Riwar, B., Baechtold, H., and Akhmedov, A.T. (1996). SMC proteins constitute two subunits of the mammalian recombination complex RC-1. EMBO J. *15*, 4061–4068.

Kerrebrock, A., Moore, D., Wu, J., and Orr-Weaver, T. (1995). Mei-S332, a Drosophila protein required for sister-chromatid cohesion, can localize to meiotic centromere regions. Cell *83*, 247–256.

Kilmartin, J., and Adams, A. (1984). Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces*. J. Cell Biol. *98*, 922–933.

Koshland, D., and Strunnikov, A. (1996). Mitotic chromosome condensation. Annu. Rev. Cell Dev. Biol. *12*, 305–333.

Lawrence, J.B., Villnave, C.A., and Singer, R.H. (1988). Sensitive, high-resolution chromatin and chromosome mapping in situ: presence and orientation of two closely integrated copies of EBV in a lymphoma line. Cell *52*, 51–61.

Li, R., and Murray, A.W. (1991). Feedback control of mitosis in budding yeast. Cell *66*, 519–531.

Lica, L.M., Narayanswami, S., and Hamkalo, B.A. (1986). Mouse satellite DNA, centromere structure, and sister chromatid pairing. J. Cell Biol. *103*, 1145–1151.

McIntosh, E.M., Atkison, M.T., Storms, R.K., and Smith, M. (1991). Characterization of a short, cis-acting DNA sequence which conveys cell cycle stage-dependent transcription in Saccharomyces cerevisiae. Mol. Cell. Biol. *11*, 329–337.

Michaelis, C., Ciosk, R., and Nasmyth, K. (1997). Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell *91*, this issue, 35–45.

Minshull, J., Straight, A., Rudner, A.D., Dernburg, A.F., Belmont, A., and Murray, A.W. (1996). Protein phosphatase 2A regulates MPF activity and sister chromatid cohesion in budding yeast. Curr. Biol. *6*, 1609–1620.

Murray, A.W., and Szostak, J.W. (1985). Chromosome segregation in mitosis and meiosis. Annu. Rev. Cell Biol. *1*, 289–315.

Nomura, N., Nagase, T., Miyajima, N., Sazuka, T., Tanaka, A., Sato, S., Seki, N., Kawarabayasi, Y., Ishikawa, K., and Tabata, S. (1994). Prediction of the coding sequences of unidentified human genes. DNA Res. *1*, 223–229.

Ohkura, H., Kinoshita, N., Miyatani, S., Toda, T., and Yanagida, M. (1989). The fission yeast *dis2*+ gene required for chromosome disjoining encodes one of two putative type 1 protein phosphatases. Cell *57*, 997–1007.

Paulson, J.R., and Laemmli, U.K. (1977). The structure of histone-depleted metaphase chromosomes. Cell *12*, 817–828.

Rao, P., and Adlakha, R. (1984). Chromosome condensation and decondensation factors in the life cycle of eukaryotic cells. Symp. Fund. Cancer Res. *37*, 45–69.

Rattner, J.B., Kingwell, B.G., and Fritzler, M.J. (1988). Detection of distinct structural domains within the primary constriction using autoantibodies. Chromosoma *96*, 360–367.

Rechsteiner, M., and Rogers, S.W. (1996). PEST sequences and regulation by proteolysis. Trends Biochem. Sci. *21*, 267–271.

Robzyk, K., and Kassir, Y. (1992). A simple and highly efficient procedure for rescuing autonomous plasmids from yeast. Nucleic Acids Res. *20*, 3790.

Rose, M.D., Winston, F., and Hieter, P. (1990). Methods in Yeast Genetics, a Laboratory Course Manual (Cold Spring Harbor, NY: Cold Spring Harbor Press).

Saitoh, N., Goldberg, I.G., Wood, E.R., and Earnshaw, W.C. (1994). ScII: an abundant chromosome scaffold protein is a member of a family of putative ATPases with an unusual predicted tertiary structure. J. Cell Biol. *127*, 303–318.

Saitoh, S., Takahashi, K., and Yanagida, M. (1997). Mis6, a fission yeast inner centromere protein, acts during G1/S and forms specialized chromatin required for equal segregation. Cell *90*, 131–143.

Saka, Y., Sutani, T., Yamashita, Y., Saitoh, S., Takeuchi, M., Nakaseko, Y., and Yanagida, M. (1994). Fission yeast cut3 and cut14, members of the ubiquitous protein family, are required for chromosome condensation and segregation in mitosis. EMBO J. *13*, 4938–4952.

Selig, S., Okumura, K., Ward, D.C., and Cedar, H. (1992). Delineation of DNA replication time zones by fluorescence in situ hybridization. EMBO J. *11*, 1217–1225.

Sikorski, R.S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics *122*, 19–27.

Straight, A., Belmont, A., Robinett, C., and Murray, A. (1996). GFP tagging of budding yeast chromosomes reveals that protein–protein interactions can mediate sister chromatid cohesion. Curr. Biol. *6*, 1599–1608.

Strunnikov, A.V., Larionov, V.L., and Koshland, D. (1993). *SMC1*: an essential yeast gene encoding a putative head-rod-tail protein is required for nuclear division and defines a new ubiquitous protein family. J. Cell Biol. *123*, 1635–1648.

Strunnikov, A.V., Hogan, E., and Koshland, D. (1995). *SMC2*, a Saccharomyces cerevisiae gene essential for chromosome segregation and condensation, defines a subgroup within the SMC family. Genes Dev. *9*, 587–599.

Sumner, A.T. (1991). Sanning electron microscopy of mammalian chromosome from pr-phase to telophase. Chromosoma *100*, 410–418.

Wilson, E.B. (1925). The Cell in Development and Heredity (New York: Macmillan Co.).

Yamamoto, A., Guacci, V., and Koshland, D. (1996a). Pds1p is required for faithful execution of anaphase in the yeast, Saccharomyces cerevisiae. J. Cell Biol. *133*, 85–97.

Yamamoto, A., Guacci, V., and Koshland, D. (1996b). Pds1p, an inhibitor of anaphase in budding yeast, plays a critical role in the APC and checkpoint pathway(s). J. Cell Biol. *133*, 99–110.

Yu, S., Ozawa, M., Naved, A., Miyauchi, T., Muramatsu, H., and Muramatsu, T. (1995). cDNA cloning and sequence analysis of novel calcium binding protein with oligoproline motif. Cell Struct. Funct. *20*, 263–268.